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GRANT NUMBER DAMD17-98-1-8576

TITLE: PTEN, a Tumor Suppressor Gene for Prostate Cancer

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REPORT DATE: July 1999

TYPE OF REPORT: Annual

PREPARED FOR: Commanding General
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	July 1999	Annual (1 Jul 98 - 30 Jun 99)

4. TITLE AND SUBTITLE
PTEN, a Tumor Suppressor Gene for Prostate Cancer

5. FUNDING NUMBERS
DAMD17-98-1-8576

6. AUTHOR(S)
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7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Baylor College of Medicine
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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

Inactivations of tumor suppressor genes are the most common genetic alterations in human cancers. The PTEN gene is a tumor suppressor gene recently cloned from human chromosome 10q23.3 that encodes a lipid phosphatase which influences a variety of cellular processes that impact on the neoplastic phenotype. The goal of this proposal is to characterize the role of the PTEN tumor suppressor gene in prostate cancer using the TRAMP mouse model, a well characterized transgenic mouse model of prostate cancer. Our research has shown that the region on mouse chromosome 19 homologous to human chromosome 10q23 is altered in a substantial fraction of TRAMP mouse prostate cancers as detected by analysis of loss of heterozygosity. We have not found a corresponding number of inactivating lesions of the retained PTEN allele, similar to the situation in clinically localized human prostate cancer as well as other human cancers such as breast cancer. We are now in a position to resolve this paradoxical observation by determining whether hemizygous loss of PTEN in itself promotes prostate tumorigenesis or whether there may be second tumor suppressor gene tightly linked to PTEN by analysis of tumor progression in PTEN heterozygote knockouts crossed with TRAMP mice

14. SUBJECT TERMS	15. NUMBER OF PAGES
Prostate Cancer	11
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

FOREWORD

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Michael Hu 7/1/99

PI - Signature Date

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INTRODUCTION

Inactivations of tumor suppressor genes are the most common genetic alterations in human cancers. The PTEN gene is a recently cloned tumor suppressor gene encoding a lipid phosphatase that acts as a counterbalance to phosphatidylinositol-3 kinase activity, and in this manner influences a variety of cellular processes that impact on the neoplastic phenotype. The goal of this proposal is to characterize the role of the PTEN tumor suppressor gene in prostate cancer using the TRAMP mouse model, a well characterized transgenic mouse model of prostate cancer, in which the SV40 T antigen is expressed under the control of the prostate specific probasin promoter (1).

BODY

Task 1: Cloning and analysis of mouse PTEN genomic clone

We have cloned the mouse PTEN gene from a SV129 phage library and have made considerable progress in defining its intron/exon structure and developing primers for mutation analysis. However, this task has a lower priority than originally thought due to our somewhat surprising finding that, in contrast to the human situation (2), the mouse genome does not contain a PTEN pseudogene. We have established this fact by a combination of Southern blotting and PCR of genomic DNA (see Appendices Figures 1 and 2). The presence of the pseudogene in humans makes mutation analysis by RT-PCR unreliable. However in the mouse this technique can be applied, and for larger tumors is the preferred technique due to its efficiency.

Tasks 2 and 4: Collection of TRAMP tumors/cell lines for PTEN analysis and analysis for PTEN inactivation

We have established a breeding colony of TRAMP mice here at the Houston VAMC that we will use for future experiments. In addition, in collaboration with Dr Craig Chenault and Dr Norman Greenberg at the Baylor College of Medicine we have collected 40 locally advanced and metastatic tumors for analysis. Such tumors are ideal for analysis due to the high purity of the tumor in these tissues, usually 80-95% cancer. Analysis has revealed that approximately 40% of these cases show loss of heterozygosity near the PTEN locus on mouse chromosome 19. However, mutation analysis by RT-PCR has failed to detect point mutations in the PTEN coding sequence. We have also analyzed these tumors by Southern blotting using Phosphorimager quantitation and controls for DNA loading and transfer and have not found evidence of homozygous deletion. We have also analyzed a subset of these tumors by Western blotting with anti-PTEN antibody and have found no loss of PTEN protein. Thus, similar to the situation in clinically localized human prostate cancer, there is a high rate of LOH near the PTEN gene but not a corresponding rate of PTEN inactivation. Two possible explanations for this phenomena are:

(1) loss of one allele of the PTEN gene is in itself sufficient to promote tumorigenesis.

(2) a second tumor suppressor gene is present near the PTEN locus.

Given that the discordance between LOH at 10q23 and inactivation of PTEN is present in a variety of human tumors (3), answering this question is of critical importance in understanding the role of alterations at 10q23 in human cancer. We have also established a total of 14 TRAMP prostate cancer cell lines for future studies.

Task 3: Creation of prostate tissue specific PTEN knockout

Given our results above we have assigned a lower priority to the generation of a tissue specific PTEN knockout until we can develop more definitive proof for the role of PTEN inactivation in prostate cancer progression in the TRAMP mouse model. Fortunately, since this grant was submitted, several groups have generated PTEN knockout mice. The homozygous PTEN knockout is an embryonic lethal but the heterozygote knockout is viable and shows a increased rate of several tumors (4). In the prostate, the heterozygote mice show proliferative/dysplastic changes of the prostatic epithelium but no invasive cancers (4). To definitively assess the role of PTEN inactivation in prostate cancer in the TRAMP model, we have obtained PTEN heterozygote knockouts from Dr Ramon Parsons at Columbia University and have established a breeding colony here at the Houston VAMC. We will breed the PTEN heterozygotes with TRAMP mice and evaluate the rate of tumor formation in the PTEN heterozygote knockout X TRAMP vs. wild type X TRAMP mice and analyze the resulting tumors for PTEN inactivation. If hemizygous loss of PTEN is sufficient to promote prostate tumorigenesis, then the PTEN heterozygote X TRAMP will develop tumors faster but will not display high rates inactivation of the retained PTEN allele. If no difference in the rate of tumor progression is seen and no inactivation of the retained PTEN allele is detected then this would be a strong indication that there is a second tumor suppressor gene tightly linked to PTEN. This will be confirmed by LOH studies of the resultant tumors in that if a second tumor suppressor gene is present, then there should be equal LOH of the PTEN region on the chromosome containing the inactivated allele and the normal allele. We also plan to continue to establish TRAMP cell lines for further studies.

Other tasks

Tasks 5-7 are planned for the second year of this grant.

KEY RESEARCH ACCOMPLISHMENTS

- Cloning and intron/exon mapping of mouse PTEN gene
- Demonstration of the absence of a PTEN pseudogene in mice
- Collection and analysis of DNAs, RNAs and proteins from TRAMP prostate cancer tumors for PTEN inactivation.
- Establishment of 14 TRAMP prostate cancer cell lines
- Establishment of TRAMP and PTEN heterozygote knockout breeding colonies

REPORTABLE OUTCOMES

- Establishment of 14 TRAMP prostate cancer cell lines

CONCLUSIONS

Our research has shown that the region on mouse chromosome 19 homologous to human chromosome 10q23 is altered in a substantial fraction of TRAMP mouse prostate cancers as detected by analysis of loss of heterozygosity. We have not found a corresponding number of inactivating lesions of the retained PTEN allele, similar to the situation in clinically localized human prostate cancer as well as other human cancers such as breast cancer. We are now in a position to resolve this paradoxical observation by determining whether hemizygous loss of PTEN in itself promotes prostate tumorigenesis or whether there may be second tumor suppressor gene tightly linked to PTEN by analysis of tumor progression in PTEN heterozygote knockouts crossed with TRAMP mice.

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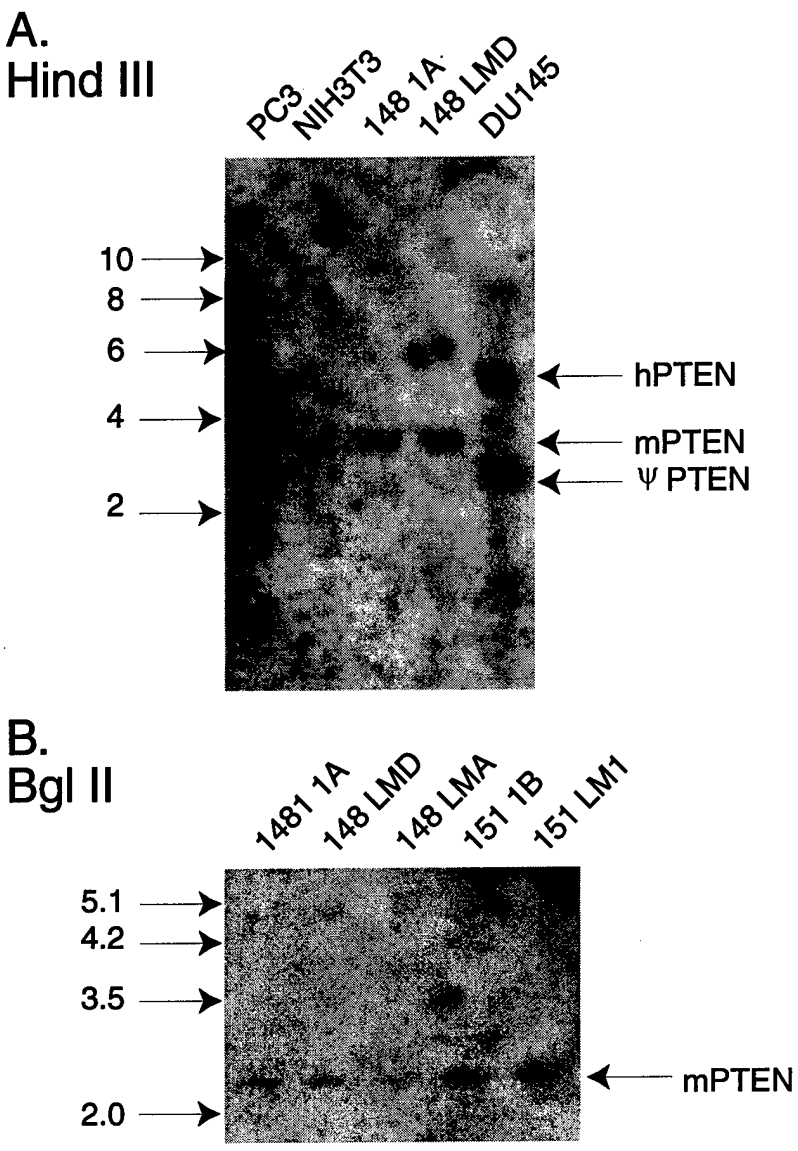
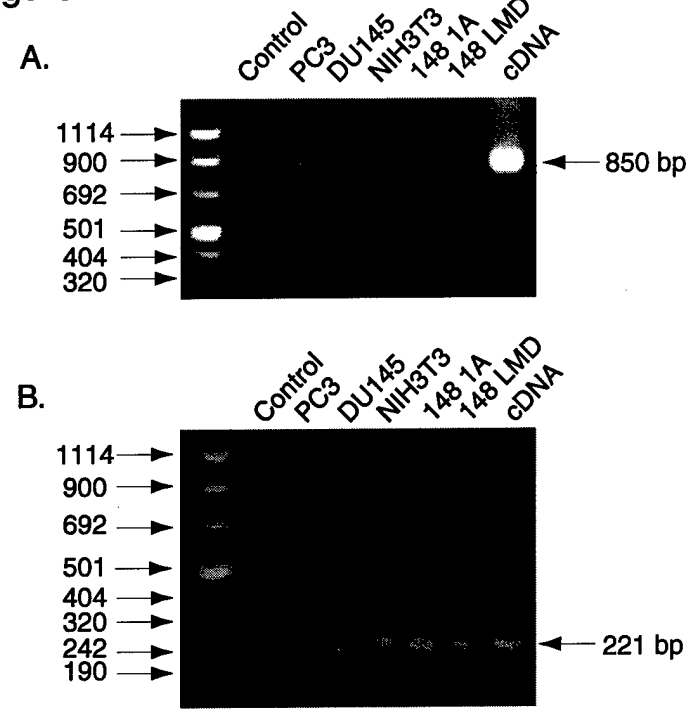
Figure 1



Human prostate cancer cell line DU145 displays two distinct bands on Southern blotting, corresponding to the PTEN gene and its pseudogene while the PC3 contains only the pseudogene due to homozygous deletion of PTEN. Using two different restriction enzymes, only a single band is identified in several mouse prostate cancer cell lines, indicating only a single PTEN gene in the mouse genome.

Figure 2



PCR with PTEN primers that span exons 4-9 gives a product with human genomic DNA corresponding to the PTEN pseudogene and with mouse CDNA but not with mouse genomic DNA (A). PCR with control primers within exon 5 are positive in all cases. These results indicate that no pseudogene is present in the mouse genome.